

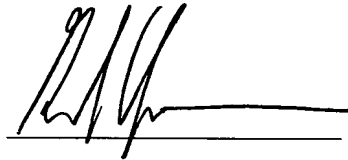
U.S. Application No. 10/069,304

July 29, 2002
Attorney Docket No. P 25,762 USA
Page 7

the specification to bring it in conformity with a Sequence Listing being submitted concurrently. In addition, Figures 1(c), 1(d), and 1(e) were added. These figures were inadvertently left out of the present application but were included with U.S. provisional Application No. 60/159,122 which is incorporated into the present application by reference (see page 48 of the present application). No new matter has been added by the present amendment.

A marked-up version of the changes made to the specification by the current amendment is attached.

Submitted respectfully,

A handwritten signature in black ink, appearing to read 'Gene J. Yao', is written over a horizontal line.

Gene J. Yao
Reg. No. 47,193
Attorney for Applicants

Synnestvedt & Lechner LLP
Suite 2600, Aramark Tower
1101 Market Street
Philadelphia, PA 19107-2950
(215) 923-4466 - Telephone
(215) 923-2189 - Facsimile

VERSION WITH MARKINGS TO SHOW CHANGES MADEIn the Description

The paragraph, commencing at page 13, line 14, has been amended as follows.

Figure 11. Shows the nucleic acid molecules of [SEQ ID NOS:6 & 7] [[SEQ ID NO.: 6]] and the amino acid sequence of [SEQ ID NO:8] [[SEQ ID NO.: 7]].

In a preferred embodiment, the figure shows the sequence of the predicted Arabidopsis gene - Accession number AAC98010

- A) Genomic Sequence [SEQ ID NO:6]. The predicted open reading frame is underlined. The start codon (ATG) and stop codon (TGA) are double underlined.
- B) Translation of the predicted open reading frame [SEQ ID NOS:7 & 8]. The transmembrane domain is underlined.

The paragraph, commencing at page 13, line 22, has been amended as follows.

Figure 12. Shows the nucleic acid molecules of [SEQ ID NOS:9 & 10] [[SEQ ID NO.: 8]] and the amino acid sequence of [SEQ ID NO:11] [[SEQ ID NO.: 9]].

In a preferred embodiment, the figure shows the sequence of the predicted Arabidopsis gene - Accession number AAD15491

- A) Genomic Sequence [SEQ ID NO:9]. The predicted open reading frame is underlined. The start codon (ATG) and stop codon (TGA) are double underlined.

B) Translation of the predicted open reading frame [SEQ ID NOS:10 & 11]. The transmembrane domain is underlined.

The paragraph, commencing at page 13, line 30, has been amended as follows.

Figure 13. Shows the nucleic acid molecules of [SEQ ID NOS:12 & 13] [[SEQ ID NO.: 10]] and the amino acid sequence of [SEQ ID NO:14] [[SEQ ID NO.: 11]].

In a preferred embodiment, the figure shows the sequence of the predicted Arabidopsis gene - Accession number CAA18823.

C) Genomic Sequence [SEQ ID NO:12]. The predicted open reading frame is underlined. The start codon (ATG) and stop codon (TGA) are double underlined.

D) Translation of the predicted open reading frame [SEQ ID NOS:13 & 14]. The transmembrane domain is underlined.

The paragraph, commencing at page 14, line 7, has been amended as follows.

Figure 14. Shows the nucleic acid molecules of [SEQ ID NOS:15 & 16] [[SEQ ID NO.: 12]] and the amino acid sequence of [SEQ ID NO:17] [[SEQ ID NO.: 13]].

In a preferred embodiment, the figure shows the sequence of the predicted Arabidopsis gene - Accession number CAA18590

A) Genomic Sequence [SEQ ID NO:15]. The predicted open reading frame is underlined. The start codon (ATG) and stop codon (TGA) are double underlined.

B) Translation of the predicted open reading frame [SEQ ID NOS: 15 & 16]. The transmembrane domain is underlined.

The paragraph, commencing at page 43, line 12, has been amended as follows.

The isolation of novel *Brassica napus* receptor kinases relied upon the newly [constructed cDNA] constructed cDNA library and involved *in vivo* mass excision of the pBluecsript phagemids from the Uni-ZAP XR vectors as outlined by the manufacturer (Stratagene, La Jolla, CA). Following efficient mass excision, phagemid DNA was extracted using a large scale alkaline protocol as described by Sambrook et al. (1989) and subjected to the polymerase chain reaction (PCR) using two separate oligonucleotide combinations, RK1/RK2 and RK1/RK3 (obtained from M. Cock, École Normale Supérieure de Lyon, France) specifically designed to prime conserved subdomains of the catalytic domain of receptor protein kinases. RK1 (5'-ggiggTTTCggiAT^Tc_AgTiTT^A_TcAA^Agg - 3'; [SEQ ID NO:18]) served as the forward primer and was constructed based upon a conserved amino acid consensus (GGFGIV^F/YKG; [SEQ ID NO:19]) within subdomain I of the catalytic domain. The degeneracy of one reverse primer RK2 (5' - AAiATiC^T_gigCCATiCC^A_gAA^A_gc - 3'; [SEQ ID NO:20]) reflects a conserved amino acid consensus (DFGMARIF; [SEQ ID NO:21]) of subdomain VII which closely resembles the SRKs in Brassica. The second reverse oligonucleotide RK3 (5' - A^g_AiA^g_AcTTigCiA^A_giCC^A_gAA^A_gTC - 3'; [SEQ ID NO:23]) was generated based upon conserved amino acids (DFGLAKLL; [SEQ ID NO:24]) within subdomain VII prevalent among the RLKs isolated in Arabidopsis. Phagemid DNA was amplified in a reaction mixture containing 1 microliter of excised phagemid DNA, 10x PCR buffer (100mM Tris-HCl pH8.3, 500mM KCl, 15mM MgCl₂), 10mM deoxyribonucleotide triphosphate mixture, 10 micromolar of each oligonucleotide primer and 0.5 microliter Tsg polymerase (BioBasics, Canada). The PCR reaction was heated at 95°C for 2 min and amplified for 35 cycles under the following amplification conditions: 1 min at 95°C for denaturation, 1 min 30 sec at 50°C for primer annealing and 1 min at 72°C for

synthesis. A final extension cycle of 10 min at 72°C was also incorporated into the amplification program. All PCR products generated of the expected size (420-450 bp) were gel purified, cloned into the pT7Blue plasmid (Novagen, Madison, WI) and introduced into *Escherichia coli* DH5- alpha. Transformants were tested for the presence of an insert and positive clones were sequenced with universal primers (R-20 and U-19) by an ABI automated sequencer (Model 373 STRETCH DNA; Perkin Elmer Corp., Canada Ltd.) using the dideoxychain-terminating method described by Sanger et al. (1977). Sequence analyses performed using DNAsis® software (Hitachi Software, San Bruno, CA) at the nucleotide and amino acid levels.

The paragraph, commencing at page 45, line 5, has been amended as follows.

The 5' end of the PERK1 cDNA was obtained by the procedure for the rapid amplification of cDNA ends originally described by Frohman et al. (1988) using the 5' RACE System, Version 2.0 kit (Gibco-BRL, Gaithersburg, MD). First strand cDNA was synthesized from approximately 300:g of mixed Westar and W1 pistil total RNA using a gene specific primer GSP1 (5'-TAACCAACAAGAgACA-3'; [SEQ ID NO:22]) designed to anneal approximately 300 bp from the 5' end of the PERK1 cDNA (1512 bp) isolated from the library screen. Following cDNA synthesis, the first strand product was purified from unincorporated dNTPs and GSP1 using a GLASS MAX® spin cartridge. A homopolymeric tail was added to the 3' end of the cDNA using TdT (terminal deoxynucleotidyl transferase) and dCTP. Tailed cDNA was amplified using a second gene specific primer GSP2 (5'-CCACTCCCAACTTTCAAC-3'; [SEQ ID NO:25]) designed to anneal 3' to GSP1 with respect to the cDNA, and an abridged anchor primer (Gibco-BRL, Gaithersburg, MD) which annealed to the homopolymeric tail. PCR amplification was carried out for 35 cycles of denaturation

at 94°C for 1 min, primer annealing at 55°C for 1 min and extension at 72°C for 2 min, followed by a final extension cycle for 10 min. A PCR product of the expected size (~1 kb) corresponding to the 5' end of PERK1 was gel purified, cloned into the pT7Blue plasmid (Novagen, Madison, WI) and transformed into *Escherichia coli* DH5- alpha. Confirmation of the 5'RACE product was obtained by plasmid Southern blot analysis as described above and by sequential primer based sequencing.

The paragraph, commencing at page 45, line 25, has been amended as follows.

A PCR based approach was used to generate a full length PERK1 cDNA by combining the 5'RACE product cloned into the EcoRV site of pT7Blue with the cDNA isolated from the library screen cloned into the EcoRI/XhoI sites of the pBluescript SK phagemid. A forward primer (5'-ggAAAgCTTgCATgCCTgCAGgTCgAC -3'; [SEQ ID NO:26]) containing an internal PstI site was designed to anneal upstream to the EcoRV cloning site of pT7Blue. A reverse primer (5'-CgCCTgCAGgTAATACgACTCACTATAggg -3'; [SEQ ID NO:27]) also containing a PstI site was designed based on pBluescript phagemid sequence immediately 3' to the EcoRI/XhoI cloning site. Full length PERK1 cDNA was generated from a 100 microliter PCR reaction containing 1 microliter (~20ng) of each template (cDNA in pT7Blue and pBluescript phagemid), 10x Pfu Buffer (200mM Tris-HCl pH8.8, 100mM (NH₄)₂SO₄, 20mM MgSO₄, 1% Triton®X-100, 1mg/mlBSA), 10mM dNTPs, 50pmol forward and reverse primers and 1microliter Pfu polymerase (Gibco-BRL, Gaithersburg, MD). The samples were heated to 94°C for 5 min and amplified for 30 cycles with a denaturing cycle of 1 min, a primer annealing cycle at 53°C for 1 min followed by an extension cycle for 3 min at 72°C. The resulting PCR product of the expected size (~2.2kb) was gel purified and cloned into the PstI restriction site of pBluescript KS (+/-) II. The full length PERK1 cDNA

U.S. Application No. 10/069,304

July 29, 2002

Attorney Docket No. P 25,762 USA

Page 13

sequence was confirmed by a sequential primer based sequencing approach using both universal and sequence specific primers as previously described. All DNA and protein sequence analysis was performed using the DNAsis[®] Software (Hitachi Software, San Bruno, CA).